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Ramona Bosch, J Proteomics Bioinform 2016, 9:11(Suppl)
http://dx.doi.org/10.4172/0974-276X.C1.092

7th International Conference on
Proteomics & Bioinformatics
October 24-26, 2016 Rome, Italy

Expression and purification of membrane scaffold proteins for the design of discoidal phospholipid bilayer nanoparticles

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The expression and purification of recombinant proteins is an everyday business. Years ago, however, protein production was difficult, time consuming and remained mostly in the domain of experts. The progress of simple, commercially available systems made the technology more widespread and led to an increase in protein production. In this work, a derivate of the human apolipoprotein A-1, the Membrane Scaffold Protein (MSP) is produced biotechnologically in a lab scale bioreactor. The MSP is a genetically engineered protein which has the notable feature to self-assemble into discoidal nanoparticles in the presence of synthetic phospholipids. These so-called nano discs have become increasingly important in the last few years e.g., for the study of membrane-associated proteins. In previous studies, nano discs are assembled by adding a micelles-organized detergent-phospholipid mixture to an aqueous system containing the MSPs. Upon removal of detergent, 10 nm diameter particles are formed. Unfortunately, the resulting nano discs are disordered in this solution and therefore further processing into an ordered and directed membrane cannot be easily achieved. The aim of this work is to create biomimetic membranes consisting of cross-linked nano discs e.g., the translocon SecYEG as part of an embedded protein complex for an active biological transport of potential target proteins. Now, the approach should be replaced by nano patterning of the nanoparticles. It is assumed that the phospholipids interact with the positive charge of a gold lamella and this effect leads to planar aligned nano discs. In next phase, the discs will be cross linked via cysteines which are located in the membrane scaffold proteins. These cysteines serve as cross-links for the disulfide bonds. Then, the gold lamella must be removed and the newly synthesized nano disc-membrane can be coated onto an ultrafiltration membrane for more stability. The resulting membrane allows for a directed investigation of the function of membrane proteins. Therefore, biomimetic membranes consisting of cross-connected nano discs have a high potential to serve as excellent biotechnological tools for research on membrane-associated proteins as well as in method development for selective separation or transport of biomolecules.

Biography

Ramona Bosch completed her studies in Biology with Specialization in Molecular Biology and Microbiology at the Karlsruhe Institute of Technology, Germany. During her Diploma thesis, she investigated the efficiency and classification of antimicrobial substances against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Subsequently, she started her Doctoral studies at the University of Hohenheim, Institute of Food Science and Biotechnology, Department of Bioprocess Engineering. Her research focusses on "The development of biotechnological processes including all process steps (upstream processing, bio-production, downstream processing) which are necessary for the industrial production of biotechnological products".

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